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Screening for sugar and ethanol processing characteristics from anatomical fractions of wheat stover

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Abstract

Due to concerns with stover collection systems, soil sustainability, and processing costs to produce ethanol, there are opportunities to investigate the optimal plant fractions to collect. Wheat stover fractions were separated by hand and analyzed for glucan, xylan, acid-soluble lignin, acid-insoluble lignin, and ash composition. Internodes had the highest glucan content (38.2% zero percent moisture basis) and the other fractions varied between 29.9% and 33.4%. The stover fractions were pretreated with either 0%, 0.4%, or 0.8% NaOH for 2 h at room temperature, washed, autoclaved, and saccharified. In addition, acid pretreated samples underwent simultaneous saccharification and fermentation (SSF) to ethanol. In general, the acid and alkaline pretreatments produced similar trends with leaves requiring very little pretreatment to achieve high conversion rates (greater than 80%). Chaff responded very well to pretreatment and high conversion efficiencies resulted when pretreated under alkaline or acidic conditions. Nodes and internodes were more recalcitrant than the other anatomical fractions. Pretreatment with 0.8% sulfuric acid (0.24 g sulfuric acid/g biomass) did not result in a significantly higher conversion of glucan to ethanol as the native material. Pretreatment with 0.8% NaOH (0.06 g NaOH/g biomass) at room temperature for 2 h resulted in high conversion efficiencies for all plant fractions, greater than 73% of the available glucan. These differences in pretreatment susceptibilities suggest that a biomass collection system that removes specific portions of wheat stover could result in significant differences in ethanol production costs.

Keywords: Biomass; Cellulase; Straw; Digestibility; Botanical; Pretreatment; Internodes; Nodes; Leaves; Chaff; *Triticum aestivum*

1. Introduction

Lignocellulosic materials represent an underutilized source of fermentable sugars. However, to convert lignocellulosic materials biologically, pretreatment is required to increase the digestibility of cellulose before conversion to glucose by enzymes [1]. Glucose can then be fermented into ethanol or numerous other chemical building blocks [2]. Hemicellulose can be converted to xylose during pretreatment and enzymatic hydrolysis and the resulting xylose fermented into valuable chemicals, if the appropriate microorganism is available.

Pretreatment and enzyme hydrolysis has been identified as one of the primary cost barriers in the processing of lignocellulosic materials to fermentable sugars [3,4]. Numerous factors affect the enzyme digestibility of cellulose such as crystallinity, accessible surface area, and protection of cellulose by lignin [1]. It would be expected that stover source and anatomical plant fraction could influence these factors. Hence, mechanical plant fractionation during collection could influence the effectiveness of dilute acid or alkaline pretreatment and other downstream processes. Papatheofanous et al. [5] found that disc milling wheat would result in two fractions: a chip fraction that was mostly internodes and a meal fraction containing primarily leaves and nodes. The internodes contained 8% more cellulose, 9% more lignin, and 10% less ash than whole wheat stover. Single- or two-stage acidic/

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aqueous-ethanol pretreatment resulted in retention of up to 99% of the cellulose and a reduction in the lignin fraction of 30%.

Acid and alkaline pretreatment of biomass has been extensively studied [1]. Dilute sulfuric acid pretreatment has been shown to effectively hydrolyze the hemicellulose and increase the enzyme digestibility of the remaining cellulose [6,7]. Chang et al. [8] found that wheat straw pretreated with 0.1 g $Ca(OH)_2$/g biomass at 120 °C for 1 h resulted in a glucan and xylan yield of 60% and 80%, respectively. Dilute acid pretreatment of wheat straw followed by simultaneous saccharification and fermentation (SSF) resulted in ethanol conversion yields of 82% when the cellulose loading was limited to 7.5% [9].

Wyman et al. [10] found that corn cobs, followed by corn stover, and wheat straw resulted in the highest concentration of ethanol after acid pretreatment and SSF. Pretreatment was performed with dilute sulfuric acid and yields of 85% were achieved following SSF from wheat straw. Glucan yields of 78% were possible using separate hydrolysis and fermentation from wheat straw.

Some composition work of wheat plant fractions has been investigated. Thompson et al. [11] reported a glucan, xylan, and ash composition from wheat straw stems of 37.2%, 22.1%, and 10.1%, respectively. Aman and Nordkvist [12] measured an ash content of 4.1, 5.5, and 10.4 for internodes, nodes, and leaves, respectively. Hess et al. [13] measured similar values for the ash content from internodes and nodes, although the ash content from leaves (15.8%) was considerably higher.

Little data are available on pretreatment and hydrolysis of plant fractions, although some work has been done on the digestibility of plant fractions by cattle. Ramanzin et al. [14] investigated the degradation of barley straw in cattle rumen. They found the degradability was greatest for leaves, followed by chaff, nodes, and internodes were the least degradable. Leaves with and without ammonia pretreatment resulted in very similar rumen digestibility. However, there was a large difference in the rumen digestibility of pretreated versus untreated internodes. Shand et al. [15] measured the rumen degradability from 12 varieties of wheat. After 48 h, the rumen degradability was 61.5%, 51.4%, 40.3%, and 33.0% from leaves, nodes, chaff, and internodes, respectively. Little data are available on differences between fractions related to sugar production for fermentation into ethanol.

Stover collection systems need to be designed to maintain a balance between biomass production and maintenance of crop residues for soil sustainability. Recent research has indicated that only 20–30% of the corn residue can be removed without deleterious effects on soil carbon levels and increased risk of soil erosion [16]. Sustainable removal rates from wheat production areas probably vary greatly, and little literature data exist on the sustainable removal rate. Nelson [17] calculated the average wheat stover that could be removed from Eastern and Midwestern States. For example, an average of 50% of the wheat stover could be removed in Ohio, while 10% or less could be removed in North Dakota, Texas, and Oklahoma [17]. The low removal rates were due to limitations associated with wind erosion requirements. Wheat stover in arid regions of the Western United States, such as Idaho, is a nuisance to producers and is often burned in the field. Much of this material could potentially be available for stover collection systems.

Feedstock properties that result in high processing costs are lignin and ash. However, lignin and ash have been shown to be important to soil sustainability. Lignin is normally associated with low degradability of forages [18] and is the slowest component to decompose in the soil [19] which could have implications for soil erosion by reducing the residue cover. The ring structures of lignin are thought to be used by some microorganisms in the synthesis of stable soil organic matter [19]. Tian and Brussaard [20] determined that crop residues with a high lignin, polyphenol, and silica content need to remain in the cropping systems to maintain the soil organic matter.

Therefore, it becomes important to remove the plant fraction with the highest fermentable sugar content. Differences in composition and pretreatment efficiency significantly impact the industrial suitability of feedstocks and the overall economics and efficiency [4]. The objectives of this study were to determine the composition and effect of pretreatment severity on wheat stover anatomical fractions, specifically chaff, leaves, nodes, and internodes.

2. Materials and methods

2.1. Sample collection

Prior to wheat planting (private farm west of Idaho Falls, ID), the field was fertilized with urea, ammonium phosphate (11-52-0), ammonium sulfate (21-0-0-24), and zinc sulfate at average rates of 318, 98, 219, 0.2 kg ha^{-1}, respectively. On April 17, 2004, immediately following fertilization of the field, Challis soft white spring wheat (WestBred, LLC, Bozeman, MO) was planted at a rate of 123 kg ha^{-1}. Prior to harvest in August 2004, hand-harvested whole-wheat plant samples (selected from random locations) were collected and transported to the University of Kentucky to be separated into grain, chaff, leaves, nodes, and internodes.

2.2. Compositional analysis

All samples were dried at 45 °C and were ground using a Wiley cutting mill (C.W. Brabender Instruments, South Hackensack, NJ) through a 2 mm circular screen and sealed in plastic bags. Total solids content, carbohydrates, acid-insoluble lignin, acid-soluble lignin, and ash [21] were measured with slight modification. UV–vis assays for measuring glucose [22] and xylose [23] concentration were

used instead of HPLC due to the large quantity of samples analyzed. Neutralizing the samples with calcium carbonate was omitted because the UV–vis assays were not affected by the acidity. Glucose and xylose concentrations were converted to the equivalent polymeric concentration (correction of 0.9 for glucose and 0.88 for xylose) and all values are reported on a zero percent moisture basis.

2.3. Alkaline pretreatment and enzyme hydrolysis protocol

Each component (3 ± 0.1 g ground through a 2 mm circular screen) was placed in a 50 ml centrifuge tube. Samples were pretreated by soaking at room temperature for 2 h in 20 ml of deionized water with 0%, 0.4%, or 0.8% NaOH (0, 0.029, or 0.058 g NaOH/g biomass). Further details about the pretreatment and enzyme hydrolysis protocol can be found in Crofcheck and Montross [24].

Enzyme hydrolysis was performed using a solution of sodium acetate (0.05 M) buffer that was autoclaved on liquid cycle for 15 min. After the solution cooled, sodium azide ($0.35\,g\,l^{-1}$) were added to control microorganisms and the pH was adjusted to 4.8 using sodium hydroxide or hydrochloric acid solutions. Enzyme loading of 0.75 g/100 ml was used for all experiments (Alltech, Inc., Nicholasville, KY) with a cellulase activity of 10,000 CMCU/g (measured at a pH of 4.8 and a temperature of 50 °C) and a xylanase activity of 150,000 XU/g (measured at a pH of 5.3 and a temperature of 50 °C). The cellulase activity in filter paper units was measured as 184 FPU/g [25]. The samples were placed in a shaking incubator (New Brunswick, New Brunswick, NJ) at a temperature of 50 °C for 65 h. Samples (5 ml) were taken at 65 h, placed in a test tube, and boiled for 5 min to inactivate the cellulase [26]. Samples (1 ml) were placed in micro-centrifuge tubes and centrifuged at 12,000 rpm for 10 min and stored at 4 °C until analysis. A Thermo-Nicolet Nexus FT-IR 670 spectrometer (Waltham, MA) with a scanning range of 400–4000 cm^{-1}, a spectral resolution of 4 cm, and 128 scans was used to simultaneously measure glucose and xylose in the enzymatically hydrolyzed samples [27].

2.4. Acid pretreatment and simultaneous saccharification and fermentation

Because an autoclave was used, only within-run experimental conditions were statistically compared. Ground biomass samples were dried overnight in the oven at 80 °C. Samples were then cooled and weighed; 2.5 ± 0.5 g (recorded to the nearest 0.0001 g) was added to a 500 ml Erlenmeyer flask. Seventy-five milliliters of 0.8% sulfuric acid (w/w) was added to the flasks and autoclaved at 121 °C, 145 kPa for 30 min. After the flasks had cooled, the wet biomass was poured through Gooch crucibles with glass filters, set in a vacuum manifold. The flasks were then rinsed with less than 20 ml deionized water to recover all the biomass. The liquid was then placed in a 100 ml volumetric flask and volume brought to 100 ml with deionized water. The biomass was then washed with deionized water for 3–4 min, weighed, and stored for use in SSF. To hydrolyze the oligomers in the pretreatment liquid, 2.95 ml was added to a test tube with 0.05 ml of concentrated sulfuric acid. This was autoclaved at 121 °C, 145 kPa for 20 min. Pretreatment liquid (2 ml) was then neutralized with 1–3 g of lead carbonate. These samples were vortexed a few times per hour until the pH was 4.5 or above. Samples were then diluted 1:10, filtered, and analyzed with HPLC for lignocellulosic sugars as previously described [11].

SSF fermentations were conducted in 60 ml serum vials under aseptic conditions as previously described [28]. All solutions were sterilized by autoclaving for 30 min at 121 °C and 145 kPa, and/or by filter sterilization (0.2 μm) prior to use. All samples and controls were performed in triplicate. To each vial was added 1 g of biomass sample (dried overnight at 80 °C), or 1 g equivalent pretreated sample (corrected for percent moisture). For dry biomass samples, 24 ml of water was added to each vial. For the pretreated biomass samples, the addition of water was adjusted for the calculated percent moisture. Therefore, 17–19 ml of water was added to each vial containing pretreated biomass samples (the average quantity of water in the pretreated samples was between 5 and 7 g of water). Thus, the final volume in experimental vials was 30 ml. The SSF conditions matched the protocol by Weimer et al. [28]. Finally, 1 ml of yeast preparation was aseptically added to each vial. This gave a final OD_{600} of 0.5 in each experimental vial. The yeast was prepared by inoculating 1 CFU of *Sacchoromyces cerevisiae* NRRL Y-2034 in a 300 ml liquid yeast peptone glucose solution (YPG; $10\,g\,l^{-1}$ yeast extract, $20\,g\,l^{-1}$ peptone, $50\,g\,l^{-1}$ glucose) in a 1 l beveled flask. The vessel was incubated aerobically overnight (19–22 h) at 30 °C and 175 rpm. The culture was pelleted by centrifugation (4500 rpm at 4 °C for 5 min), followed by one wash with PBS buffer. The washed yeast pellet was resuspended in PBS to an OD_{600} of 15. The vials were then analyzed for ethanol by injecting 200 μl of the headspace gas for gas chromatographic analysis with a flame ionization detector (GC-FID). Standards were prepared using aqueous concentrations of ethanol to eliminate ethanol partitioning problems and the need to use Henry's law. Ethanol was quantified on days 1, 3, 5, and 7 then frozen to be available for continued analyses if necessary. The ethanol concentrations were normalized to the theoretical production of 0.51 g ethanol/1.0 g of glucose for graphical presentation and subsequent statistics. The total mass loss of the sample measured after pretreatment plus the relative loss of sugars were used to calculate the compositional values for expressing the SSF analysis. A positive control using corn stover that had been processed by a dilute acid pretreatment pilot scale run at NREL was run in triplicate alongside the experimental stover fractions.

Table 1
Distribution (%, w/w, zero moisture basis) of the fractions and standard error (%, w/w, zero moisture basis) determined by hand separation from five random locations within the field

Fraction	Distribution (%)	S.E. (%)
Internodes	33	0.60
Chaff	24	0.93
Leaves	18	0.72
Nodes	13	0.56
Other[a]	12	1.02

[a]Particles too small to efficiently sort into leaves or chaff.

3. Results

3.1. Composition

The distribution and standard error of wheat stover components is shown in Table 1. Overall, the total biomass yield estimated from the sample plots was 4.02 t ha^{-1} with a standard error of 0.045 t ha^{-1}. The internodes were the single largest fraction, accounting for 33% of the dry stover weight. Chaff, leaves, and nodes accounted for 24%, 18%, and 13%, respectively. The "other" category comprised 12% of the stover weight and was primarily composed of leaves and chaff that were difficult to identify because of the small particle size. It was assumed that the other category was 50% leaves and 50% chaff in future calculations.

Compositional analysis was performed on the individual wheat stover fractions and is summarized in Fig. 1. The average is based on four replicates measured from five locations within the field. Based on the compositional analysis, 80%, 82%, 83%, and 86% of the mass of nodes, leaves, internodes, and chaff, respectively, are accounted for in Fig. 1. Using a Student's *t*-test at the 95% confidence level, the glucan contents of all fractions were statistically different. Xylan was significantly lower in the internodes relative to the other plant fractions. However, the internodes were the highest in glucan. The acid-insoluble lignin was significantly different between all plant fractions, except between the internodes and nodes. Likewise, the ash contents of the plant fractions were significantly different between all plant fractions, except between the nodes and internodes. In addition, the effect of plant location within the field on the amount of glucan, xylan, lignin, and ash was significant. These differences confirmed the fact that five random samples from the field were sampled and the composition determined. Even with there being a significant difference in location, the differences due to component were more significant for each component, as shown in Table 2, which shows the *F* statistics from ANOVA (considering all two-way interactions).

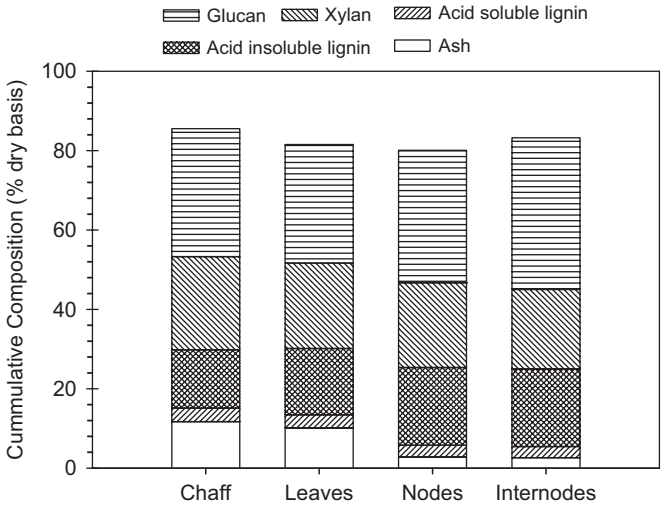

Fig. 1. Average glucan, xylan, acid-insoluble lignin, acid-soluble lignin, and ash content of wheat stover fractions measured from five locations and four replicates.

Table 2
ANOVA table with the *F* statistic calculated based on the effect of location and effect of plant fraction ($n = 5$ and four replicates)

	Location *F* statistic	Component *F* statistic
Glucan	15.2	248
Xylan	44.8	53.9
Insoluble lignin	28.5	820.4
Soluble lignin	17.1	65.5
Ash	147	4150

3.2. Alkaline pretreatment and enzyme hydrolysis results

3.2.1. Alkaline pretreatment effects

After the samples were pretreated, they were filtered and washed. The filtrate recovered was measured for glucose and xylose content using UV–vis absorbance and FTIR assays. Both measurements returned similar values, with the glucan released during pretreatment accounting for less than 0.008 g glucan/g biomass. Since all wheat stover fractions had a glucan content greater than 0.29 g/g biomass, the glucan hydrolyzed during pretreatment was less than 3% of the total available glucan. Xylan released during pretreatment was less than 0.007 g xylan/g biomass from a total available of at least 0.20 g xylan/g biomass. Changes in lignin content attributed to pretreatment were less than 2% of the total. Pretreatment conditions used in the current study were intentionally mild and limited the amount of sugar hydrolyzed and delignification during pretreatment compared to previous studies.

3.2.2. Enzyme hydrolysis following alkaline pretreatment

The cellulase loading was relatively high (46 FPU/g biomass), resulting in complete hydrolysis after 65 h and ensuring the reaction was not enzyme limited. The data reported for the enzymatic saccharification neglected the sugars released during pretreatment.

The glucan released (Fig. 2a) and the total amount of glucan recovered (Fig. 2b) from the wheat stover fractions after enzymatic hydrolysis was significantly affected by the

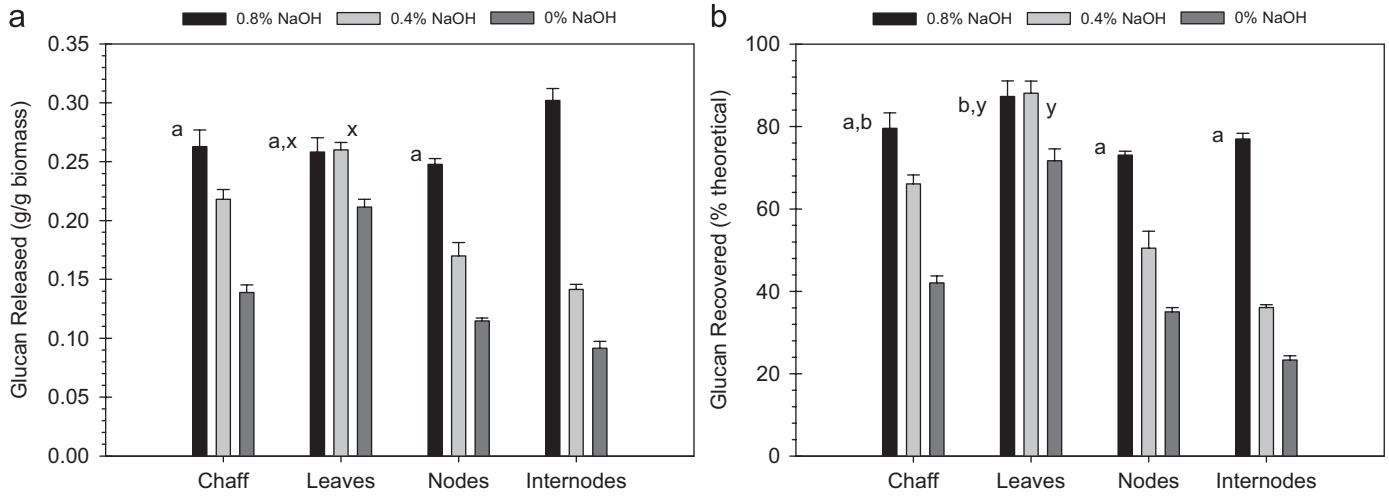

Fig. 2. Glucan converted (g/g biomass) (a) and percentage recovered (b) after pretreatment with 0%, 0.4%, and 0.8% NaOH after 65 h of enzymatic hydrolysis. Error bars represent the standard error. Data with the same letter are statistically ($\alpha = 0.05$) not different, with letters a–d denoting a comparison within pretreatments and letters x–y denoting a comparison within fraction.

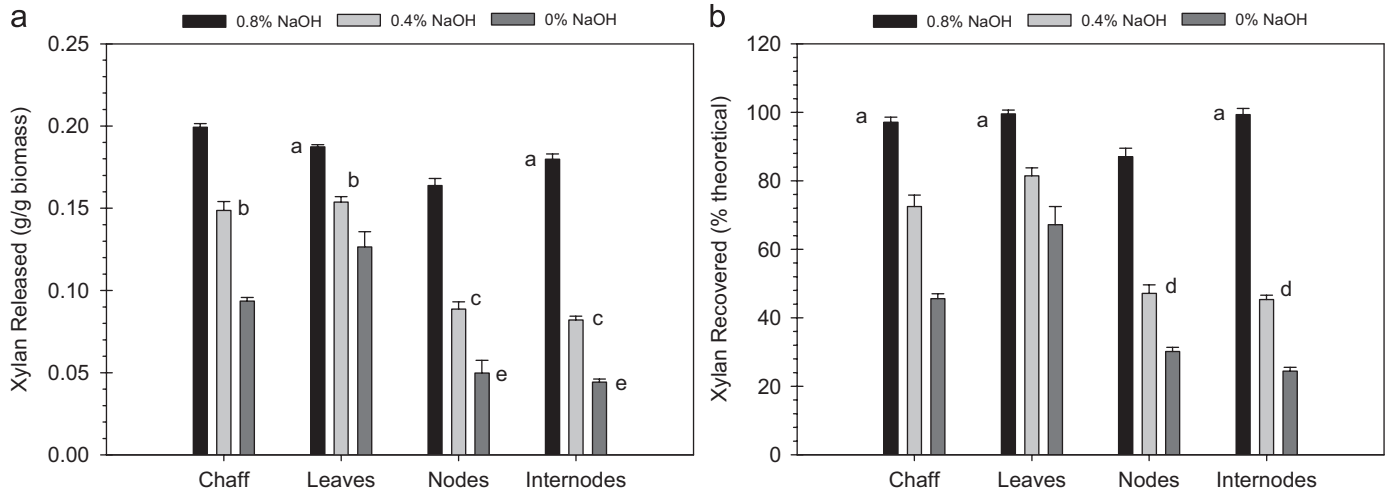

Fig. 3. Xylan converted (g/g biomass) (a) and percentage recovered (b) after pretreatment with 0%, 0.4%, and 0.8% NaOH after 65 h of enzymatic hydrolysis. Error bars represent the standard error. Data with the same letter are statistically ($\alpha = 0.05$) not different, comparisons within each pretreatment.

pretreatment severity and type of fraction. The glucan recovered was calculated as a percentage of the total glucan (Fig. 1) determined for each fraction. Internodes released the most glucan (0.30 g/g biomass) when pretreated with 0.8% NaOH. Chaff, leaves, and nodes pretreated with 0.8% NaOH released between 0.25 and 0.26 g glucan/g biomass. Interestingly, leaves released an equal amount of glucan when pretreated with 0.4% and 0.8% NaOH (0.26 g glucan/g biomass), which is attributed to the susceptibility of the leaves to pretreatment relative to the chaff, nodes, and internodes. With 0% NaOH, leaves released 0.21 g glucan/g biomass. Pretreating chaff and leaves with 0.8% NaOH resulted in 80% or more of the glucan being recovered after enzyme hydrolysis. Pretreating internodes and nodes with 0.8% NaOH resulted in 77% and 73% of the glucan being recovered, respectively.

Similar trends were observed in the quantity of xylan released (Fig. 3a) and the percentage recovered (Fig. 3b). Chaff and leaves were the fractions that released the most xylan (over 0.19 g/g biomass) when pretreated with 0.8% NaOH. Internodes and nodes released 0.18 and 0.16 g xylan/g biomass when treated with 0.8% NaOH that was slightly less than the other components. As seen with glucan, the amount of xylan released from leaves approached a maximum more quickly over the range of pretreatments than the other components. All (>97%) of the xylan was recovered from chaff, internodes, and leaves when pretreated with 0.8% NaOH. When nodes were pretreated with 0.8% NaOH, 87% of the xylan was recovered. Nearly 70% of the xylan was recovered when leaves were pretreated with 0% NaOH water, while less than 45% of the xylan was recovered when

Table 3
HPLC analysis of sugars in liquid hydrolysates from dilute acid pretreatment of wheat stover anatomical fractions

Plant fraction	Statistic	Glucan	Xylan	Galactan	Arabinan	Mannan
Chaff	Mean ± SD	4.03 ± 0.79 A	14.5 ± 0.07 A	0.37 ± 0.00	2.07 ± 0.02 B	0.00 ± 0.00
	Percentage of original polymer	10.89	56.9	nd	nd	nd
Leaves	Mean ± SD	1.80 ± 0.47 B	12.0 ± 0.61 B	0.37 ± 0.01	2.17 ± 0.16 AB	0.04 ± 0.06
	Percentage of original polymer	5.13	48.9	nd	nd	nd
Nodes	Mean ± SD	1.67 ± 0.54 B	10.0 ± 0.47 C	0.25 ± 0.06	2.59 ± 0.36 A	0.12 ± 0.12
	Percentage of original polymer	4.23	41.2	nd	nd	nd
Internodes	Mean ± SD	1.30 ± 0.54 B	9.98 ± 0.47 C	0.00 ± 0.06	1.03 ± 0.36 C	0.09 ± 0.12
	Percentage of original polymer	2.86	43.7	nd	nd	nd

Means are given as the percent of the initial sample dry mass, SD is one standard deviation. Identical letters within a column represent no significant difference observed at $\alpha = 0.05$. Galactan and mannan concentrations were considered too low for reasonable statistical application, and were not determined in the original compositional analysis (nd = not determined).

chaff, internodes, and nodes were pretreated with 0% NaOH.

3.3. Acid pretreatment and simultaneous saccharification and fermentation

With respect to the original amount of polymer present, the percent of glucan lost during the acid pretreatment averaged 6% over all fractions, while the amount of xylan lost averaged over all fractions was 48% (Table 3). The concentration of galactan, arabinan, and mannan in the original stover samples was not determined. The highest amount of glucan was removed from the chaff (11%). Xylan removal by the acid pretreatment was significantly higher in the chaff (57%) and was lowest in the nodes and internodes (41% and 44%, respectively). The galactan, arabinan, and mannan were not normalized to the initial concentration of the polymers. However, the total amount of mass removed during acid pretreatment is shown in Table 3.

Theoretical ethanol production after acid pretreatment and SSF varied greatly depending on the plant fraction (Fig. 4). The native (no sulfuric acid) chaff, node, and internode plant fractions resulted in the same theoretical ethanol yield of less than 17% and were statistically the same. However, native leaves resulted in a theoretical ethanol production greater than 76%. Pretreatment followed by SSF did not significantly increase the ethanol production from nodes and internodes and remained below 41%. Chaff responded positively to pretreatment and the theoretical ethanol production increased from 16% to 82%. Pretreated leaves were statistically the same as native leaves.

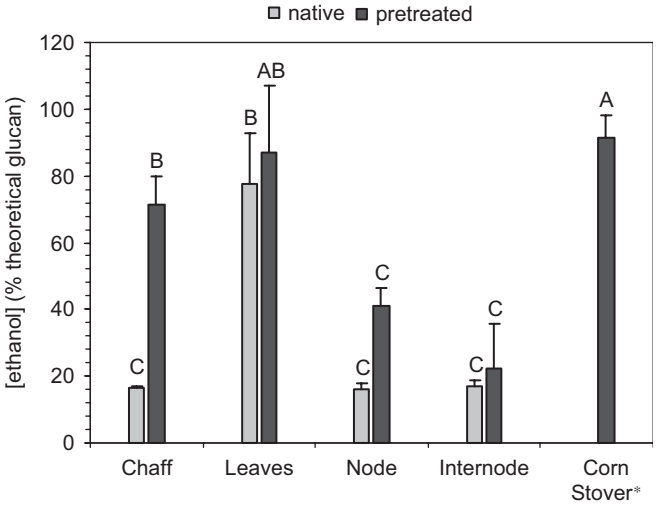

Fig. 4. SSF conversion to ethanol; day 7 ethanol concentrations in dilute acid pretreated and non-pretreated (native) corn stover anatomical fractions. Error bars represent the standard error, identical letters indicate no significant difference was observed at $\alpha = 0.05$. *Pretreated corn stover is an independent stover sampling, processed through a "full-pretreatment" dilute acid pretreatment pilot scale run, and was included as a positive control material for evaluation of the SSF run.

4. Discussion

4.1. Composition

Shand et al. [15] found the average weight distribution from internodes, chaff, leaves, and nodes in 12 wheat varieties to be 47%, 14%, 34%, and 6%, respectively. The difference in the weight distribution from the components in the two studies was probably due to the method used to separate the whole plant into fractions. In our study, the head of the plant was removed by hand and passed through a laboratory thresher. The non-grain material separated by the thresher was classified as chaff. Due to the number of samples, the nodes were broken off from the plant by hand. This resulted in the nodes having additional weight due to small pieces of the internodes being connected. Compositional data for the plant fractions were similar in this study and previously reported results. Whole stover was not directly measured, but was calculated based on the distribution of stover and compositional analysis. The whole stover from this study was 33.4%, 21.4%, 17.2%, 3.1%, and 7.1% glucan, xylan, acid-insoluble lignin, acid-soluble lignin, and ash, respectively.

4.2. Alkaline pretreatment and enzyme hydrolysis

The mass weighted average of glucan released from whole stover pretreated with 0% NaOH (deionized water) was 0.14 g glucan/g biomass. Pretreating whole stover with 0.8% NaOH resulted in 0.27 g glucan/g biomass. Glucose produced after enzymatic hydrolysis from steam-exploded wheat straw ranged from 0.23 to 0.38 g glucose/g biomass depending on the pretreatment severity and enzyme loading [29]. This compares favorably with the whole-wheat straw weighted average production of 0.31 g glucose/g biomass measured in this study. Whole stover released 0.08 and 0.18 g xylan/g biomass when pretreated with 0% NaOH and 0.8% NaOH.

An interesting observation from Figs. 2 and 3 was the linear response in sugar released after enzymatic hydrolysis resulting from pretreatment at the three levels. Glucan hydrolysis followed a linear relationship ($r^2 > 0.91$) to pretreatment level, except for leaves due to the higher hydrolysis efficiency at a pretreatment level of 0.4% NaOH. Xylan hydrolysis also followed a linear relationship ($r^2 > 0.9$) with pretreatment level, except for the leaves.

4.3. Acid pretreatment and simultaneous saccharification and fermentation

The pretreated corn stover from an NREL pilot run pretreatment resulted in the same theoretical ethanol production as the pretreated leaves. The ethanol production from the NREL pretreated corn stover was 91% of theoretical which indicated that the SSF conditions were appropriate. Surprisingly, the data seem to indicate that wheat internodes require a higher severity pretreatment than the NREL pretreated corn stover, a similar result determined by Wyman et al. [10]. This difference could be related to the structure of the plant, ash and/or lignin content of wheat internodes relative to corn stover.

4.4. Collection of optimal plant fractions

The major costs for a biorefinery are feedstock collection, storage, transportation, and pretreatment [4], hence, optimizing these unit operations throughout the entire system should prove beneficial. Increasing the available sugars of the feedstock at a fixed pretreatment level might result in reduced capital and operating costs due to decreased demand for feedstock and/or higher ethanol yield.

If low severity pretreatments are desired, chaff and leaves will produce the greatest quantity of sugar and ethanol. Overall, the biomass yield was 4.02 t ha^{-1} and over half (54%) of the material was chaff, leaves, and other particles too small to identify. This feedstock would have a glucan concentration of approximately 31.2%, or 676 kg glucan ha^{-1}. Alkaline pretreatment with 0.4% NaOH would convert approximately 75% of the glucan and acid pretreatment followed by SSF would yield an ethanol conversion that was 78% of theoretical. Assuming the fermentation efficiency for either system was 80%, the ethanol yield from the chaff and leaves was 367 l ha^{-1}. If higher severity pretreatments are used, the nodes and internodes would be more attractive. The glucan concentration of the feedstock would be 36.6%, or 677 kg glucan ha^{-1}. However, the mild pretreatment conditions used in this study would only result in a fermentation efficiency of approximately 40% for a total ethanol yield of 138 l ha^{-1}. More severe pretreatment conditions would be required to increase the fermentation efficiency. Clearly, harvest of specific anatomical fractions of wheat could yield large differences in processing efficiency and therefore cost.

Collecting material that requires a high or low severity pretreatment could be accomplished with existing harvest equipment. During harvest, combines produce chaff (primarily composed of chaff and leaves) and straw streams (internodes and nodes) that each represents approximately 50% of the total biomass yield. If 50% of the biomass could be removed in Ohio [17], the chaff and leaves would be preferable to collect to allow for a greater residue cover to limit water erosion. In arid regions where biomass is frequently burned, harvesting the internodes may be most desirable since the chaff and leaves would readily break down in the field. Chaff and leaves have an ash and lignin content of approximately 10.8% and 15.0%, while internodes and nodes have an ash and lignin content of approximately 2.6% and 19.5%. The two streams would represent a high ash/low lignin or low ash/high lignin feedstock that would probably influence the decision to use acid or alkaline pretreatment. Previous research has shown that lignin and ash are required for soil sustainability, although which component is more valuable to soil sustainability is unknown.

5. Conclusions

The glucan composition of internodes (38.2%) was substantial and was present in the largest quantity (33% of the stover) prior to grain harvest. The leaves, chaff, and nodes had glucan contents between 29.9% and 33.4%. The xylan contents of all anatomical fractions varied between 20.1% and 23.5%. The anatomical fractions behaved very differently when pretreated. Leaves required very little pretreatment and released 0.26 g glucan/g stover when pretreated with 0.4% NaOH, for a conversion efficiency of 88%. Similar results with dilute acid pretreatment followed by SSF were observed with the leaves requiring very little pretreatment. The native material resulted in 76% of the theoretical glucan being converted to ethanol and pretreatment with sulfuric acid did not significantly increase the conversion efficiency. The nodes and internodes required a more severe pretreatment level to hydrolyze the glucan and xylan. Acid pretreatment followed by SSF under the conditions investigated did not significantly increase the ethanol yield from nodes and internodes.

Wheat stover collection needs to be balanced with soil sustainability issues. Assuming that only a fraction of the

stover can be removed, optimization of collection systems to selectively harvest individual stover fractions probably will be advantageous. This would allow for a biorefinery to select feedstock fractions to minimize pretreatment and enzymatic hydrolysis demands, thereby reducing ethanol production costs, and allowing increases in the allowable purchase price for the feedstock. In turn, increasing the allowable purchase price of feedstocks significantly increases the regionally available tonnages. For example, to attain sustained biomass removal in some areas, due to regional climates and cropping practices, larger grower payments are needed for soil amendments, water applications, and feedstock supply chain costs. In such areas where feedstock costs are higher, the selective harvest strategies described herein may help provide for the economic margins desired for full-scale implementation of lignocellulosic using biorefineries.

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